Cell lineage distribution atlas of the human stomach reveals heterogeneous gland populations in the gastric antrum

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ABSTRACT

Objective The glands of the stomach body and antral mucosa contain a complex compendium of cell lineages. In lower mammals, the distribution of oxyntic glands and antral glands define the anatomical regions within the stomach. We examined in detail the distribution of the full range of cell lineages within the human stomach.

Design We determined the distribution of gastric gland cell lineages with specific immunocytochemical markers in entire stomach specimens from three non-obese organ donors.

Results The anatomical body and antrum of the human stomach were defined by the presence of ghrelin and gastrin cells, respectively. Concentrations of somatostatin cells were observed in the proximal stomach. Parietal cells were seen in all glands of the body of the stomach as well as in over 50% of antral glands. MIST1 expressing chief cells were predominantly observed in the body although individual glands of the antrum also showed MIST1 expressing chief cells. While classically described antral glands were observed with gastrin cells and deep antral mucous cells without any parietal cells, we also observed a substantial population of mixed type glands containing both parietal cells and G cells throughout the antrum.

Conclusions Enteroendocrine cell lineages show distinct patterns of localisation in the human stomach. The existence of antral glands with mixed cell lineages indicates that human antral glands may be functionally chimeric with glands assembled from multiple distinct stem cell populations.

INTRODUCTION

Among gastrointestinal tissues, the gastric mucosa is constructed from a more complex set of short lived and long lived cell lineages.1–3 While studies over the past 20 years have detailed the origin and distribution of cell lineages in the rodent stomach,1–5 far fewer studies have addressed the distribution of lineages in the human stomach. The rodent stomach is divided into three discrete anatomical zones, including the squamous lined forestomach, the glandular–oxyntic (body) region containing acid secreting parietal cells and pepsinogen secreting chief cells, and the antrum containing gastrin cells.4 In all rodents studied as well as other mammalian species, the border between the antrum and the body contains areas of transitional glands with fewer parietal cells before the initiation of antral mucous glands that lack parietal cells.6 7 It is thought that the boundaries between these regions are dictated by the developmental expression of specific transcription factors, especially sex determining region Y-box 2 (SOX2) and pancreatic and duodenal homeobox 1 (Pdx1).8 9 Thus Pdx1 expression defines the region of rodent antrum and deletion of Pdx1 causes failure of antrum formation.10

In contrast with mice, the geographic anatomy of the human stomach is far less detailed. The human stomach does not have a squamous forestomach region but rather is divided anatomically into three regions: a proximal peri-oesophageal cardia, the glandular body and the antrum.11 Traditionally, these regions have been grossly defined by the positions of the nerves of Latreille. While much of the present literature suggests that human gastric lineages are distributed in a manner similar to that in rodents and other animals, few studies have previously defined in detail the distribution of cell types within the human stomach.
We have now evaluated the geographic distribution of cell lineages within the human stomach by quantitative determination of cell numbers throughout the gastric mucosa from non-obese organ donors. These results have revealed that cell lineages are not uniformly distributed throughout the stomach and that there are distinct and important differences in humans compared with other species. Enteroeendocrine cell lineages were concentrated in discrete regions within the proximal and distal stomach. Ghrelin and gastrin best defined the anatomical body and antrum, respectively. Importantly, parietal cells were distributed in glands throughout the human antrum. Indeed, the human antrum appears to contain three distinct types of glands distributed in glands throughout the human antrum. These distributions were evaluated across all three stomach body regions with a relatively even distribution throughout the anatomical body of the stomach. Serotonin cells were far less numerous throughout the stomach body regions with a relatively even distribution throughout the anatomical body of the stomach. Chromogranin A positive cells were numerous and uniformly distributed throughout the anatomical body of the stomach defined by the ghrelin expressing cells. Significantly lower numbers of chromogranin A immunoreactive cells were also seen in the anatomical antrum. We next determined the distribution of serotonin expressing enterochromaffin cells in the stomach. Serotonin cells were far less numerous throughout the stomach, usually fewer than 10 cells per core. Compared with other markers, there was more variability in both serotonin cell numbers and distribution. Similar to findings in a previous report, all three donor stomachs showed a concentration of serotonin cells in the antrum (Figures 2D and 4D). However, we

METHODS
See online supplementary methods.

RESULTS
Mapping the geographic distribution of lineages in the human stomach
To map the distribution of cell lineages within the human stomach, the entire stomach was obtained from three organ donors (see online supplementary table S1). The stomachs were opened along the greater curvature and fixed overnight in formalin. The specimens were then divided into regions of 0.5 cm height×2 cm wide (Figure 1). Each region was separately embedded in paraffin with the specimen oriented to display glands along their length. Cores from these blocks (1 mm) were then excised and arrayed into tissue arrays that together covered the entire stomachs from the three specimens. We then stained these arrays with antibodies against specific markers of cell lineages (see online supplementary table S2). The tissue arrays were analysed by digital quantitation using an Ariol SL-50 system (Leica) and lineage abundance was quantified as cells per 1 mm core (Figure 1). The cell numbers were then displayed in two-dimensional projections of maps of the stomach specimen (see online supplementary figures S1–S3), and heat map colouration was developed to display the data. Finally, the two-dimensional maps were rendered onto three-dimensional projections of the stomach to display the distribution of lineages throughout the stomachs. Data for all three of the donor stomachs are shown in the online supplementary materials as two-dimensional maps (see online supplementary figures S1–S3). Three-dimensional rendering was performed only for donor No 2 stomach as a representative of our findings (Figures 1 and 3). Dynamic rotating reconstructions of the three-dimensional renderings are included in the online supplementary videos 1–9. To analyse the distribution of lineages in the stomachs, each specimen was divided into three body zones and antrum (see Figure 1) and the distributions of lineages were analysed as a per cent of total labelled cells (Figure 4).

Distribution of enteroendocrine cells in the human stomach
Traditionally, the gastric regions of the human stomach have been divided into body versus antral regions. We therefore examined the distribution of enteroendocrine cell lineages in the glands of these regions. As noted in numerous previous investigations, gastrin expressing cells were confined to the distal stomach (Figures 2A and 4A). In contrast, also as previously reported, ghrelin expressing cells were essentially excluded from the distal stomach and were abundant in the stomach body regions with a relatively even distribution throughout the body mucosa (Figures 2B and 4B). A similar staining pattern was also observed for obestatin, a splice variant of the ghrelin gene (data not shown). This inverse relationship between gastrin and ghrelin expression was observed in all three of the donor stomachs (see online supplementary figures S1 and Figure 4A,B). These findings suggested that ghrelin and ghrelin cells define the anatomical division between the human stomach body and antrum, respectively.

Figure 1 Procedure for geographic mapping of the cell lineages within the human stomach. The three entire donor stomachs were divided into regions of 0.5 cm height×2 cm wide to embed in paraffin blocks, and 1 mm cores from the paraffin blocks were excised and assembled into tissue arrays. These arrays were stained with cell lineage specific antibodies, and then the cell numbers per core were determined using a digital quantitation system (Ariol SL-50). Finally, the distribution of cell lineages was displayed in three-dimensional (3D) projections of the stomach.
also observed a concentration of serotonin cells in the proximal stomach.

To complete the analysis of enteroendocrine cells in the human stomach, we examined the distribution of somatostatin immunoreactive D cells. D cells were distributed throughout the body and antrum (figure 2E). In all three stomachs, there were significantly higher numbers of somatostatin cells in the proximal regions of the body mucosa (figure 4E). Thus the inhibitory influences of somatostatin cells are concentrated in the proximal stomach.21

In addition to the endocrine cell lineages, we also evaluated the presence of tuft cells in the normal human stomachs. Tuft cells are sensory mucosal cell lineages that form direct synapses with interneurons in the gastric wall.5 Staining for DCLK1, a marker of tuft cells, showed staining of rare individual cells in cores in the body and antrum (see online supplementary figure S4B). The tufts cells showed the characteristic morphology with a prominent apical extension (see online supplementary figure S4B). The tuft cells were extremely rare and no more than 20 tufts cells were identified in an entire set of stomach specimen cores.

**Distribution of mucous and secretory lineages in the human stomach**

We next examined the distribution of cells considered components of oxyntic glands: surface mucous cells, parietal cells,
chief cells and mucous neck cells. We did not strictly quantify surface mucous cell numbers because staining for either MUC5AC or diastase resistant Periodic acid–Schiff was so intense and the cell borders were difficult to discern (data not shown). Online supplementary figure S4A demonstrates that MUC5AC staining surface cells were prominent in cores from both the body and antral regions, but the length of foveolar regions was greater in the antral cores.

We evaluated the presence of proliferating cells using Ki-67 staining (figure 3A). As expected, in the body of the stomach, Ki-67 expressing progenitor cells were located in the upper gland region deep to the foveolar cells. Similarly, in the antrum, Ki-67 expressing cells were present in the mid-gland deep to the foveolar cells. Overall, analysis of the distribution of Ki-67 expressing progenitor cells demonstrated a relatively uniform distribution of proliferative cell numbers throughout the gastric mucosa (figure 4F).

H/K-ATPase staining, as expected, demonstrated large numbers of parietal cells throughout the anatomical body in all three stomachs, with 95% of parietal cells found within the body mucosa (figures 3B and 4G). However, we also found that all three stomachs showed prominent numbers of parietal cells in groups of glands in the antral region. While the numbers of parietal cells in antral glands represented only 5% of the total number in the stomach, they were consistently present in the antrum extending towards the pyloric junction (see online supplementary figure S3). The distribution of MIST1 immunoreactive chief cells followed a pattern similar to that seen for parietal cells, with 91% of cells found in the anatomical body (figures 3C and 4H). However, we again observed chief cells in groups of glands throughout the anatomical antrum.

Identification of mucous neck cells is predicated on the use of antibodies against MUC6 or its companion trefoil protein, TFF2. However, MUC6 and TFF2 are also secreted from the

Figure 3 Geographic mapping of progenitor and oxyntic gland cell lineages. Tissue array sections were stained for (A) Ki-67 (progenitor cells), (B) H/K-ATPase (parietal cells), (C) MIST1 (chief cells) and (D) MUC6 (mucous neck cells and deep antral gland cells). Quantitated cell lineage numbers per core were mapped onto three-dimensional stomach maps to demonstrate the distribution of cells in the stomach (left panels). The scale bars represent the quantitated range of positive cells in a core. Representative cores from the body and antrum are shown at the right of each panel with high magnification insets showing the individual cell staining pattern. In the antral core for MUC6 staining in (D), insets show staining for cells with mucous neck cell morphology on the left, and cells with deep antral gland cell morphology on the right.
deep antral gland mucous cells as well as from Brunner’s glands. Thus, as expected, we observed the presence of MUC6 expressing cells throughout the stomach (figures 3D and 4I). In the fundic region, MUC6 immunoreactive cells displayed a small triangular cell morphology and were present in the mid-gland region, all characteristics of mucous neck cells (figure 3D). However, in the antral region, cells with two different morphologies were observed. Some glands showed the morphology of mucous neck cells in the mid-gland regions but others showed the foamy, open ended morphology classically ascribed to deep antral gland mucous cells (figure 3D). Thus together these data suggested that oxyntic type glands were present throughout the human antrum.

The human antral mucosa is assembled from a mixture of ‘oxyntic’ and ‘gastrin’ glands

To analyse in greater detail the structure of glands in the antrum, we performed dual staining for H/K-ATPase and gastrin on arrayed samples of the stomach and paraffin sections of human antrum from six other organ donor specimens. Figure 5 demonstrates that we observed a marked heterogeneity among gland types in the anatomical antrum, with groups of glands containing gastrin cells (figure 5D) interspersed with glands containing parietal cells (figure 5C). Most of the antral cores where gastrin cells were present showed heterogeneity among the glands. In addition, 50% of glands showed a ‘mixed’ phenotype containing both gastrin cells and parietal cells (figure 5B). These mixed-type glands contained fewer parietal cells per gland compared with the oxyntic glands in the body, and the parietal cells were generally located deep to the gastrin expressing cells. Within these glands, we occasionally observed cells, which co-stained for both H/K-ATPase and gastrin (see online supplementary figure S5A). These findings suggest that H/K-ATPase and gastrin expressing cells in the antrum might be derived from the same progenitor cell population.

Characterisation of antral gland subtypes

To examine the relationship of these three gland types in the distal human stomach, we stained 13 serial sections from the human antrum for gastrin and H/K-ATPase along with p120 to outline the lateral membranes of cells (figure 5E and see online supplementary figure S5B). The images of the stained serial sections were then used to assemble three-dimensional reconstructions of the gland structures (see online supplementary video 10). The reconstruction shows that the gland types, while separated in the deep portions, merged with each other in the upper foveolar regions (see online supplementary figure S5B). Furthermore, foveolar regions often displayed further ramifications. These results suggest that the deep antral glands form with multiple lineage configurations.

As the oxyntic-type glands in the antrum clearly contained only a third the number of parietal cells observed in the oxyntic...
glands in the stomach body, we next sought to determine if these glands contained other lineages traditionally observed in oxyntic glands. We utilised the morphology of TFF2 expressing cells to assess the presence of mucous neck cells. Figure 6A demonstrates that parietal cell containing glands also contained mucous neck cells. These cells were clearly distinguishable as small triangular cells compared with the larger basal antral gland-type cells. We also stained for MIST1 expressing chief cells, which are derived from mucous neck cells. MIST1 expressing chief cells were also present in glands with parietal cells, but not in glands with gastrin cells (figure 6B).

Immunohistochemical studies had demonstrated that the antral glands were essentially devoid of ghrelin cells, indicating that there were differences in the antral oxyntic glands. Histamine secreting ECL cells perform a central role in the stimulation of acid secretion by parietal cells. A previous investigation had demonstrated that ECL cells are present throughout the human stomach. We therefore sought to determine whether the antral glands with parietal cells also contained histamine secreting ECL cells by staining for histidine decarboxylase. Histidine decarboxylase immunoreactive cells were observed throughout the antrum, and dual labelling studies showed that ECL cells were present in all three types of antral glands (see online supplemental figure S6).

Previous investigations in rodents have suggested that antral gland cells express the transcription factor Pdx1, which is responsible for patterning in the distal foregut. We therefore stained human antral samples for Pdx1 to determine whether the parietal cells in the antrum also express this antral marker (figure 6C). Pdx1 expressing cells were observed in glands with gastrin cells but not in glands with only parietal cells. Pdx1 co-labelled with gastrin cells in the ‘mixed’ glands. However, Pdx1 was not observed in the nuclei of H/K-ATPase expressing parietal cells, suggesting that Pdx1 expressing antral progenitor cells may not give rise to parietal cells in the mixed glands.

Figure 5 Immunofluorescence staining for parietal cells in human antrum. Paraffin sections of human gastric antrum were immunostained for H/K ATPase (H/K) (parietal cells, green) and gastrin (GAS) (G cells, red). P120 (grey scale) immunostaining was used for lateral membrane staining and 4’,6-diamidino-2-phenylindole (DAPI) (blue) was used for nuclear staining. Dotted boxes in (A) indicate regions enlarged in (B–D). Three populations of glands were observed in the human antrum: (B) oxyntic glands with parietal cells but not gastrin cells, (C) mixed glands with both parietal cells and gastrin cells and (D) antral-type glands with gastrin cells. Scale bars are as indicated. (E) Tracings of glands in 13 serial sections. Gland morphologies were defined as ‘gastrin’ or ‘mixed’ based on triple labelling with antibodies against gastrin (red), H/K-ATPase (green) and p120 (blue). The colour coding for traced gland units was as follows: red, orange and green, antral-type glands lacking parietal cells; pink and yellow, mixed-type glands with both parietal cells and gastrin cells; and light blue and blue, incompletely mapped glands.
Putative gastric stem cells in the human antrum

To understand how the mixture of cell lineages is assembled in antral glands, we next examined the distribution of putative gastric stem cells. In a mouse study, SOX2 expressing stem cells in the stomach were found in both the body and antrum, and the SOX2 expressing stem cells gave rise to both oxyntic and parietal cells.

Figure 6 Characterisation of gastric glands in human antrum. Paraffin sections of human gastric antrum were immunostained for trefoil factor 2 (TFF2) (A), MIST1 (B) or pancreatic and duodenal homeobox 1 (Pdx1) (C) with both gastrin and H/K-ATPase. 4',6-Diamidino-2-phenylindole (DAPI) was used for nuclear staining. In (A), the mucous neck cells (white arrows) and the deep antral mucous cells (yellow arrows) were immunostained for TFF2. In (C), gastrin expressing G cells were co-localised with Pdx1 (white arrows), however, H/K-ATPase expressing parietal cells were not co-labelled with Pdx1 (yellow arrows). Dotted boxes depict regions enlarged. Scale bars are as indicated.
antral gland lineages.25 We observed SOX2 expressing cells in the antral specimens of the human stomachs. SOX2 expressing cells were widely distributed throughout all three types of glands (see online supplementary figure S7A). Some SOX2 expressing cells were located between parietal cells and gastrin cells, and we observed that 1.95% of cells were co-positive for both SOX2 and H/K-ATPase and 2.50% were co-positive for both SOX2 and gastrin (see online supplementary figure S7A, white arrows and see online supplementary figure S7C). We also stained for Ki-67 to assess whether the SOX2 expressing cells were proliferative (see online supplemental figure S7B). We observed that the Ki-67 positive progenitor cells were located adjacent to SOX2 expressing cells. However, SOX2 expressing cells did not co-label with Ki-67. Thus these data suggested that the SOX2 expressing cells may represent a candidate of putative quiescent stem cells in the human antrum, which can give rise to both ‘oxyntic’ and ‘antral’ gland lineages.

**DISCUSSION**

The present results have defined in detail for the first time the geographic anatomy of cell lineages within the human stomach. The findings here indicate that enteroendocrine cells are not, in general, uniformly distributed in the human stomach. Indeed ghrelin and gastrin are the best markers of the anatomical body and antrum, respectively. A concentration of somatostatin cells was observed in the proximal stomach. Previous studies have noted the enrichment of ghrelin cells in the human body of the stomach.16 Other studies have suggested that large numbers of enteroendocrine cells are present in the fetal human stomach before lineages such as parietal cells and chief cells that develop late in gestation.26 Thus given the extensive projections that are present for most enteroendocrine cells,27 it is tempting to suggest that enteroendocrine cells may be a critical influence for the differentiation of gastrointestinal lineages during development as well as during adult life.28 29 Nevertheless, it is interesting to note that these antral oxyntic glands, unlike their counterparts in the body, do not contain any ghrelin cells. Mixed glands have both parietal cells and gastrin cells, but do not contain chief cells.

Previous investigations in animal models have emphasised developmental regional borders within the gastrointestinal tract that define the boundaries of mucosal lineage derivation.10 Thus the antrum and glandular body in rodents contain gastrin or parietal cells, respectively. While transitional glands are present between the rodent body and antrum,6 they do separate homogeneous regions of oxyntic glands versus antral mucous glands. The derivation of the antrum in the mouse correlates with the domain in the distal foregut for expression of the regulatory transcription factor Pdx1.10 Glands in the antrum of rodents appear to be derived from Lgr5 expressing stem cells38 whereas the glands of the fundic mucosa are not derived from such cell populations.39 Our present findings suggest that the human antral mucosa is often made up of a mixture of oxyntic-type and antral-type glands. We have also identified a population of glands with mixed lineages (figure 7). In addition, occasional cells were observed expressing both H/K-ATPase and gastrin (see online supplementary figure S7). These findings indicate that a different paradigm must exist to explain the mixed gland phenotype in humans. The SOX2 expressing stem cells that are considered as marking quiescent stem cell populations are present in the mixed glands in human antrum. Thus in humans, these SOX2 expressing stem cells may be able to give rise to both gastrin cells and parietal cells. Alternatively, mixed glands may contain two distinct sets of progenitor cells necessary for generating either parietal cells or gastrin cells. Nevertheless, in the context of the mixed glands, these progenitors appear to generate not only all of the gastrin gland lineages (surface cells, gastrin cells and deep antral mucous cells), but also parietal cells and ECL cells, without producing other normal oxyntic lineages (mucous neck cells and chief cells) (figure 7). Nevertheless, the presence of other glands that are completely oxyntic or completely antral indicates that specialisation is also possible. Wright et al have previously noted that glands may replicate by fission to yield patches of glands derived from a single founder gland.40 As the range of plasticity is not clear, it is not possible to determine whether mixed glands represent the origin of these patches of oxyntic versus antral glands.
Taken together, these data suggest that there is considerable acid secretory capacity in the antrum from humans. However, it remains unclear why, with the notable exception of the Tominaga paper, most recent authors have only commented on the presence of a smaller number of parietal cells in the transition zone between the body and the antrum. Tominaga noted no overall difference in the distribution of parietal cells in the antrum of patients with gastritis. We have examined three complete stomachs and six antral specimens from organ donors, none of whom showed *H pylori* infection. It is therefore possible that some of the differences may relate to the influence of *H pylori* on antral gland lineages. However, as Tominaga’s observations were made in the 1970s when *H pylori* infection was extremely common in Japan, this possibility seems less likely. Thus while the inbred rodent strains used in most research may have more uniform patterns of gland geography, humans seem to possess a range of gland derivation patterns in the antrum. It is possible that these differences are related to genetic backgrounds, as examination of human fetal stomachs showed considerable heterogeneity in the distribution of parietal cells in the antrum. In our own work, we have found no evidence for age related effects on patterns of parietal cell distribution in the antrum. Thus the human population seems to manifest considerable heterogeneity in the presence of mixed and oxyntic glands within the antrum.

In earlier studies in humans and rodents, we have demonstrated that spasmolytic polypeptide expressing metaplasia (SPEM) is associated with local focal changes stemming from parietal cell loss. These changes often involve only single glands, leading to the suggestion that SPEM reflects a normal reparative response to local damage of gastric glands. Indeed, we did observe instances of single SPEM glands lying within normal mucosa in the donor stomach samples (see online supplementary figure S8). In our previous investigations, we have focused on metaplasia in the fundic region of the stomach, because it was difficult to identify morphologically the presence of SPEM in the antrum where the deep antral gland cells have similar morphology and express similar markers (MUC6 and TFF2) as SPEM cells. Nevertheless, our findings that the human antrum has a mixture of gland types raises the question of the glandular origin of intestinal metaplasia in the human antrum. Previous investigations have noted that gastrin cells are completely absent in glands with intestinal metaplasia in the antrum. Thus it is possible that intestinal metaplasia (as well as SPEM) might arise from oxyntic glands within the human antrum. This concept would provide a unified explanation for metaplastic processes in the stomach.

In summary, the present investigations demonstrate that a complete examination of the distribution of lineages within the human stomach has revealed complexity or heterogeneity in lineage distribution in the human antrum compared with lower mammalian species. The presence of three discrete types of glands within the human antrum suggests that the pattern of lineage derivation in the distal human stomach is more complicated than that detailed in rodent models. We have also documented regional concentrations of enteroendocrine cells within the stomach. Taken together, these findings indicate that geographic distributions of cell lineages and gland configurations within the human stomach may contribute to key aspects of gastric physiology and pathophysiology.

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Contributors EC and JTR: designed and performed the studies, analysed the data and drafted the manuscript. BJB, RO and AER: performed the studies. CS: designed the studies and analysed the data. JRG: designed the studies, analysed the data and drafted the manuscript.

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Competing interests None.

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METHODS

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Characteristics of human tissue samples

Entire stomachs were obtained from three non-obese individuals at the time of organ harvest under an IRB-approved protocol that was designated as non-human subject research. The characteristics of the subjects are detailed in Supplemental Table 1. All three donor stomach samples were negative for *H. pylori* by CLO test on antral mucosa. Donor specimens were opened along the greater curvature of the stomach, fixed for a minimum of 10 hours in 10% buffered formalin, and submitted for standard processing for FFPE tissue. Each specimen was dissected into 3 mm rows that were further divided into 2 cm width length segments. These segments were mapped onto the original specimen to allow for precise localization. Each block of stomach tissue was examined with H&E stain for orientation of the mucosal glands. Areas of mucosa with optimal orientation from each block were selected for 1 mm core excision and arraying into tissue microarrays (TMA). A single tissue core was selected from block. TMAs were constructed for each donor stomach in the Vanderbilt Translational Pathology Shared Resource.

For correlative studies of antral gland anatomy, we used antral sections from six further non-obese human donors all of whom were *H. pylori* negative.

Immunoctaining.
Human stomach sections were deparaffinized and submitted to antigen retrieval in a pressure cooker using the Target Retrieval solution (Dako North America, Inc., Carpinteria, CA). Primary antibody incubation was performed overnight at 4 °C. For immunohistochemistry, secondary antibody incubation and DAB development were performed using the Dako Envision+ System-HRP DAB according to the manufacturer’s instructions. In the case of immunofluorescence, appropriate secondary antibodies conjugated with Alexa 488, Cy3, or Cy5 were used (1h incubation at room temperature).

In some cases, for dual labeling using two rabbit primary antibodies, we employed the TSA (Tyramide Signal Amplification)-Plus Fluorescein or Cyanine 3 Kits (PerkinElmer) to detect the first primary antibody of each pair (used in this case at a dilution 10-20 fold higher than in a regular staining). After washes with 1X PBS and a second blocking step, we performed the incubation with the second primary antibody of each pair, which was then detected using Alexa 488- or Cy3-conjugated secondary antibodies. In both cases, we included a control slide for which the second primary antibody of each pair was omitted. Detailed information on primary antibodies and their dilutions are listed in Supplemental Table 2.

**Tissue array imaging and quantitation.**

Immunostained tissue microarray slides were imaged on the Ariol SL-50 automated slide scanner (Leica). Slides were imaged at 20X magnification to a resolution of 0.323 μm/pixel. Cells were identified in each core by utilizing standard Ariol analysis scripts. Upper and lower thresholds for color, saturation, intensity, size, roundness, and axis length were set for both blue Hematoxylin staining of nuclei and for brown DAB reaction
products. Thus, brown (DAB) positive cells can be distinguished from blue (Hematoxylin only) negative cells. Cell numbers were recorded for each core and the data were imported into Microsoft Excel to aide in the creation of 3-dimensional maps of each staining pattern.

To analyze the distribution of cell lineages with the stomachs, we defined a zone in the distal stomach as antrum based on the distribution of gastrin cells. We then defined three zones in the remaining body region by dividing the distance of the remaining specimens in thirds based on the cephalo-caudal axis (see Figure 1). The percent of cores in each zone were as follows: Donor 1: Body 1, 24%; Body 2, 31%; Body 3, 27% and Antrum, 18%. Donor 2: Body 1, 27%; Body 2, 32%; Body 3, 26% and Antrum, 15%. Donor 3: Body 1, 29%; Body 2, 33%; Body 3, 25% and Antrum, 13%.

For each of the labeled cell lineages the distribution was defined by determining the number of cells in each zone as a percent of the total number of labeled cells in the entire specimen. These percentages were then compared across all three specimens using an ANOVA with post-hoc comparison of significant means with Bonferroni’s test (p<0.05 for significance).

3-Dimensional rendering of stomach staining.

The numbers of cores representing each stomach were so great that they needed to be spread over multiple slides. Thus, for each immunostain tested between 2 to 5 slides were analyzed for each stomach. Data obtained from the analysis of tissue microarray cores for each stomach and stained marker were pooled into master lists in Microsoft Excel. Separately, 2-dimentional maps were created in Adobe Illustrator CS6 to characterize the
original layout of each stomach and specifically identify the origin of each core sample. Following Ariol analysis, the numbers of positive cells identified in each core were translated onto the 2-dimentional maps in Adobe Illustrator. A “heat map” of color ranges from solid blue to solid red was created to represent the range of positive cells for each immunostained marker. Basically, the natural log of each cell count present in each stomach was listed in ascending order. This order was then assigned a value using the following equation where A equals the natural log of the cell count:

\[
\text{Hue} = \text{ROUND}((240-(A2*(240/(\text{MAX(OFFSET($A$1,1,0,COUNTA(A:A),1))-MIN(OFFSET($A$1,1,0,COUNTA(A:A),1))))),0))
\]

The values produced by this equation were then utilized to create HSB (Hue, Saturation, Brightness) color swatches where the Hue value was variable between 240 and 0, while the Saturation and Brightness values remained 100%. Each cell representing an individual cell count in each 2-dimentional map was colored with the HSB value corresponding to cell count (Supplemental Figures 1 and 2). Colored 2-dimentional stomach maps were then stretched into square Mercator projections of themselves in order to create image maps for 3-dimentional modeling. A wireframe general stomach model was created in the professional modeling software Cinema 4D (MAXON Computer GmbH, Germany) based on rough axis ratio measurements of human stomachs. The 2-dimentional Mercator projections for each stomach were then “wrapped” around this 3-dimentional wireframe model. Wrapped models were then rendered and animated in Cinema 4D to produce tiff images and Quicktime movies.

**Three-dimensional reconstruction of human antral glands.**
Thirteen serial 5 µm human stomach sections were cut from a gastric antrum block. Immunolabeling was performed using the same procedure as above in the immunohistochemistry protocol. Briefly, the Gastrin primary antibody (at a dilution 1:8,000) was incubated overnight at 4 °C, then the Gastrin primary antibody was detected using TSA (Tyramide Signal Amplification)-Plus Cyanine 3 Kit. After the second blocking step using Protein Block Serum-Free solution, the H/K-ATPase and P120 antibodies were incubated at 4 °C for overnight, which were then detected using Alexa448- and Cy5-conjugated secondary antibodies with DAPI staining of nuclei.

The serial sections were imaged with the Ariol SL-50 automated slide scanner at 20X magnification to a resolution of 0.323 µm/pixel. A matching, aligned, representative area was extracted from each of the serial sections and saved in Tagged Image File Format (TIFF). Individual stomach glands were traced out using in Adobe Photoshop CS6 (Adobe), using the anti-p120 and DAPI staining as guides. The traces were then reassembled into a 3-dimentional object using the Imaris software package (Bitplane/Andor Technology).
Supplemental Figures

Supplemental Figure 1: Two-dimensional maps of cell numbers from all three donors for endocrine lineages.

A. Gastrin

B. Ghrelin

C. Chromogranin
Supplemental Figure 2: Two-dimensional maps of cell numbers from all three donors for cell lineages.

D. Serotonin

E. Somatostatin

F. Ki67
Supplemental Figure 3: Two-dimensional maps of cell numbers from all three donors for cell lineages. Two-dimensional maps from all three donors with quantitation of G) H/K-ATPase (parietal cells), H) Mist1 (chief cells) and I) MUC6. The two-dimensional maps for donor 2 correspond to the 3-dimensional maps shown in Figure 3.
Supplemental Figure 4: Staining of representative cores from the body and antrum.

A

H&E

MUC5AC

BODY

B

H&E

MUC5AC

ANTRUM

DCLK-1

ANTRUM
Supplemental Figure 5: A. Immunofluorescence staining for H/K-ATPase and Gastrin co-positive cells. Immunostaining for H/K-ATPase and gastrin from the stomach antrum demonstrates the presence of an H/K-ATPase and gastrin co-positive cell (yellow arrow). B. Assembly of gland tracing. Upper tracings of individual glands were outlined based on stained images in the lower panel for triple labeling with antibodies against gastrin (red), H/K-ATPase (green) and p120 (blue). The color-coding for traced gland units was as follows: Red, orange, and green – antral-type glands lacking parietal cells, pink and yellow – mixed-type glands with both parietal cells and gastrin cells, light blue and blue – incompletely mapped glands.

Supplemental Figure 6. Immunofluorescence staining for ECL cells in the human antrum.
Supplemental Figure 7: Immunofluorescence staining for SOX2-expressing gastric stem cells in human antrum. Paraffin sections of human gastric antrum were immunostained for SOX2, gastrin and H/K-ATPase (A) and for SOX2 and Ki67 (B). DAPI was used for nuclear staining. Dotted boxes depict regions enlarged. White arrows indicate the position of cells co-labeled for both H/K-ATPase and SOX2. Scale bars are as indicated. C. Quantitation of SOX2-expressing cells co-positive for H/K-ATPase or Gastrin in human antrum. Sections were triple stained for SOX2, gastrin and H/K-ATPase. The graph represents the proportion of SOX2-expressing cells co-positive for SOX2 and gastrin (SOX2(+)GAS(+)) or co-positive for SOX2 and H/K-ATPase (SOX2(+)H/K(+)) or singly positive only for Sox2 (SOX2(+)) per 100 SOX2-expressing cells (n=3).
Supplemental Figure 8: Immunostaining for MUC6 in a core from the stomach body shows a single SPEM gland. Immunostaining for MUC6 in this core from the stomach body demonstrates the normal distribution of MUC6-staining mucous neck cells. The arrow indicates the presence of one gland with MUC6 staining of cells throughout a gland to its base, characteristic of SPEM.
**SUPPLEMENTAL TABLE 1.** Characteristics of three human organ donors whose stomachs were studied for lineage geography.

<table>
<thead>
<tr>
<th>Donor</th>
<th>AGE</th>
<th>SEX</th>
<th>WEIGHT (lb)</th>
<th>HEIGHT (in)</th>
<th>BMI</th>
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<tr>
<td>1</td>
<td>57</td>
<td>Female</td>
<td>140</td>
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<td>Male</td>
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<td>70</td>
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<td>21</td>
<td>Male</td>
<td>176</td>
<td>76</td>
<td>21.4</td>
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<td>MARKER</td>
<td>LINEAGE</td>
<td>SOURCE</td>
<td>DILUTION</td>
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<td>----------------------------------</td>
<td>---------------------------------------------</td>
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<tr>
<td>H/K-ATPase</td>
<td>Parietal cells</td>
<td>Fitzgerald, 10R-H100b</td>
<td>1:10,000 or 1:50,000</td>
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<tr>
<td>Mist1</td>
<td>Chief cells</td>
<td>Gift from Jason Mills, Wash. U., St. Louis</td>
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<td>Muc5AC</td>
<td>Surface cells</td>
<td>NeoMarkers, MS-551-P</td>
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<td>MUC6</td>
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<td>Kanto Chemical, 25503-96</td>
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<td>Enteroendocrine cells</td>
<td>AbD Serotec, MCA845</td>
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<tr>
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<td>D cells</td>
<td>DAKO, A0566</td>
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<td>EC cells</td>
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<td>Gastrin</td>
<td>G cells</td>
<td>BioGenex, AR019-5R</td>
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<td>Ghrelin</td>
<td>X cells</td>
<td>Phoenix Pharmaceuticals, H-031-31</td>
<td>1:10,000</td>
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<td>Histidine decarboxylase</td>
<td>ECL cells</td>
<td>Fitzgerald, 20R-HP001</td>
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<td>Ki-67</td>
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<td>Gastric stem cells</td>
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**SUPPLEMENTAL TABLE 2.** List of primary antibodies used in this study with sources and working dilutions.